

## Correspondence

## Placozoa – no longer a phylum of one

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More than a century ago, the simplest of all metazoans was discovered and described as *Trichoplax adhaerens* [1]. These tiny, flattened animals lack symmetry, mouth, gut, nervous system, and extra-cellular matrix and constitute the apparently monotypic phylum Placozoa. Placozoans diverged early in metazoan history [2–7], making them important organisms for evolutionary research [2,3,8]. Placozoans can be found in warm, shallow, marine environments around the world [9] and all observed individuals fit the general morphological description of *T. adhaerens*. Our analyses, however, show that the phylum Placozoa is significantly more diverse than previously thought.

Extensive genetic variation in Placozoa is revealed (Figures 1 and 2) by four molecular markers: the small and large ribosomal

subunits (SSU and LSU), the internal transcribed spacers 1 and 2 and ribosomal 5.8S (ITS), and the large subunit of the mitochondrial ribosome (16S). With SSU, for example, the genetic distances between individual placozoans are comparable to those documented between genera (within families) and even between families (within orders) of other diploblastic, early diverging metazoan phyla (Supplemental Data). Moreover, from only 31 placozoans sampled around the world (Table 1), we obtained eight different haplotypes of mitochondrial 16S (Figure 2), displaying length variation of up to 145 bp, a level far exceeding that documented for any metazoan species or genus. The inferred secondary structures for 16S are correspondingly diverse (Figure 2).

Tree topologies inferred from all four markers are largely congruent (Figures 1 and 2). Intra-individual variation in ITS is low relative to the divergences between the five deeper divisions indicated by both 16S and ITS (Figure 2). These data strongly reject the idea that the phylum Placozoa is represented by a single extant species. Thus, caution is warranted in interpreting comparative studies that use a single clonal lineage. In addition, the combined SSU and LSU data (Figure 1), as well as two other recent studies [7,10], convincingly refute the view of

placozoans as degenerate medusozoan cnidarians [11].

We have advanced in our understanding of placozoan biodiversity, but much further investigation of these intriguing animals is needed. The species richness of Placozoa is still to be determined. More importantly, placozoans have been studied mostly in the laboratory and nearly all observations have been made on a single clonal lineage, the Grell culture-strain originating from the Red Sea (H1). The importance of studying animals living in their natural habitat is undeniable: a complete reproductive cycle has never been reported in all the decades placozoans have been kept in the laboratory.

Based on limited sampling to date, H6, 7 and 8 are exclusively Pacific, and H1, 2, 3 and 5 are not found in the Pacific (Table 1). Otherwise, our data have so far displayed little biogeographic signal. Several placozoan lineages show a widespread and overlapping geographic distribution while several locales have yielded multiple sympatric lineages. A general correlation between small body size and high abundance suggests that microbial eukaryotes (< 1 mm) may be less likely to exhibit biogeographic patterns because their large populations experience few dispersal barriers [12]. When captured in the field on settling

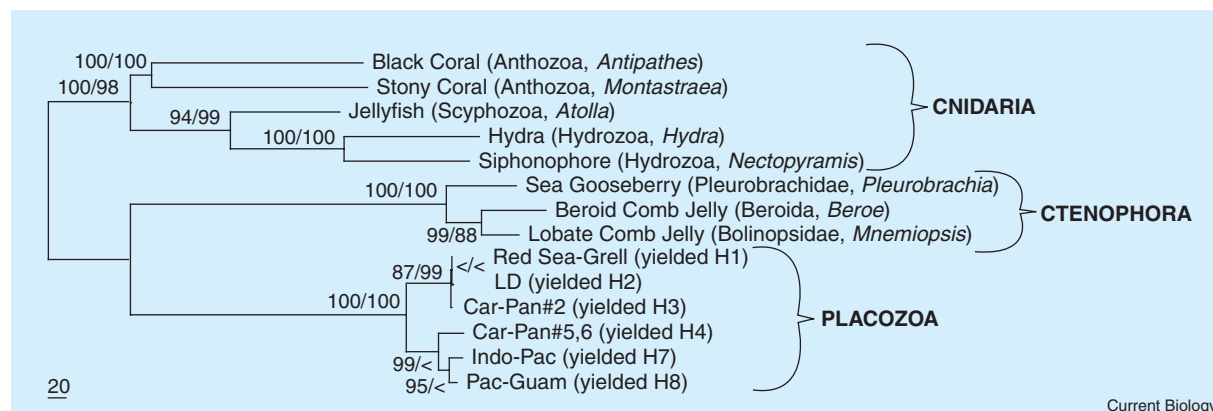


Figure 1. Parsimony phylogram of placozoans based on combined SSU (18S) and LSU (28S) data.

The tree is rooted with disparate cnidarians and ctenophores (Accession numbers: *Antipathes*, AF100943, AY026365; *Montastraea*, AY026382, AY026375; *Atolla*, AF100942, AY026368; *Hydra*, AF358080, AY026371; *Nectopyramis*, AF358068, AY026377; *Pleurobrachia*, AF293677, AY026378; *Beroe*, AF293694, AY026369; and *Mnemiopsis* AF293700, AY026373), and with bootstrap indices under parsimony (1000 replicates) and likelihood (100 replicates). '<' indicates a bootstrap value of less than 50. Assumed model of nucleotide evolution for likelihood analyses: one rate for transversions, two rates for transitions, proportion of invariant sites, and shape parameter (TrN+I+G). The 16S haplotype derived from each of the samples is shown in parentheses. Scalebar: 20 character changes.

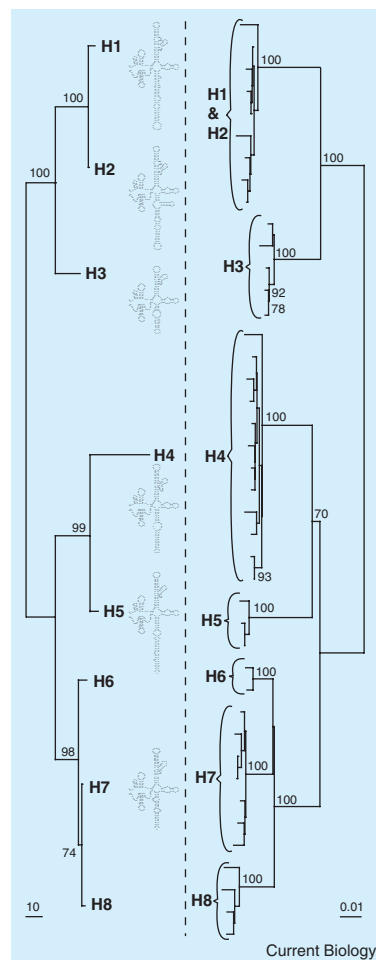


Figure 2. Correspondence of relationships based on 16S (left) and ITS (right). Left: MP phylogram of eight 16S haplotypes obtained from 31 samples (Table 1), with bootstrap indices (1000 replicates). Icons representing differing secondary structures are shown for six of the haplotypes. Scalebar: 10 character changes. Right: ME phylogram of ITS, with bootstrap indices (200 replicates) exceeding 69. Assumed model of nucleotide evolution: four rates for transversions, one rate for transitions, and gamma shape parameter (TVM+G). Scalebar: 0.01 substitutions per site.

slides, placozoans are only ~100–200  $\mu\text{m}$  across, so they could fit this model. Another possible explanation for weak biogeographic patterning are widespread anthropogenic introductions. Indeed, both natural dispersal and artificial transport may contribute to the distribution of placozoans.

Our data provide the beginnings of a systematic hierarchy for Placozoa. However, placozoan diversity is currently best classified simply by referring to

Table 1. Origin of samples, listed by 16S haplotype.

H1:	Red Sea (Grell Clone, $n = 1$ ); Caribbean (Panama, $n = 1$ )
H2:	Caribbean (Panama, $n = 2$ ); Mediterranean (Italy, $n = 4$ ); Unknown (Aquarium, $n = 1$ )
H3:	Caribbean (Panama, $n = 1$ )
H4:	Caribbean (Panama, $n = 3$ ; Venezuela, $n = 1$ ); Pacific (Panama, $n = 5$ )
H5:	Mediterranean (Italy, $n = 3$ )
H6:	Pacific (Panama, $n = 2$ )
H7:	Pacific (Panama, $n = 1$ ); Indo-Pacific (Aquarium, $n = 1$ ); Unknown (Aquaria, $n = 2$ )
H8:	Pacific (Guam, $n = 1$ ; Panama, $n = 2$ )

the well-supported clades (Figure 2). When the full life cycle and the extent of morphological and ecological variation are documented, a systematic classification — free from historical bias — can be erected for this entire phylum.

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#### Supplemental data

Supplemental data are available at <http://www.current-biology.com/cgi/content/full/14/2/2/R944/DC1/>

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